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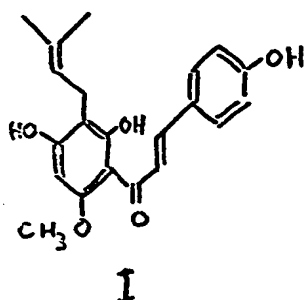
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- (72) Inventors WILLIAM MITCHELL, ANTHONY MARTIN
 HUMPHREY and JOHN MICHAEL FINCHER

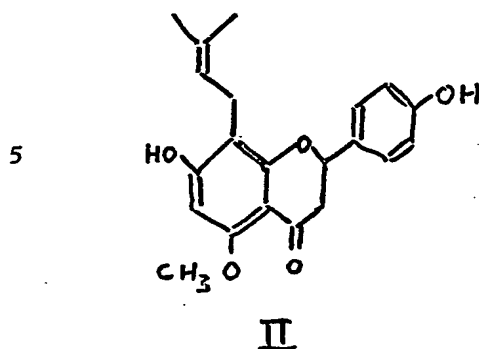


(54) HOP EXTRACTS

- (71) We, BUSH BOAKE ALLEN LIMITED, a British Company of 20/42 Wharf Road, London, N.1, England, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—
- The present invention relates to the preparation of hop extracts.
- Hops comprise resins, oils, waxes, water-soluble materials (sugars, tannins and proteins) and a cellulosic matrix. It is primarily the resins and oils which account for the value of the hop to the brewers.
- The resins may be subdivided into hard resins, which are sparingly soluble in hydrocarbon solvents and are regarded as being of minor significance in brewing, and the soft resins. The latter include two important groups of weakly acidic compounds called the α -acids and β -acids respectively. The α -acids, which are the more acidic of the two groups, are known generically as the humulones and are converted by boiling with wort to iso α -acids, which are primarily responsible for the bitter flavour of beer. The β -acids, known generically as lupulones, contribute to a lesser extent to the bitterness of beer.
- The oils include the volatile essential oils, which are of importance to the aroma and quality of beer, and relatively involatile fixed oils, which are contained in the hop seeds and are not normally present in beer.
- It has long been recognised that the traditional brewing method of boiling hops with wort prior to fermentation is very inefficient with regard to the utilisation of the hop resins. In particular only a small proportion of the α -acids from the hop appear as iso α -acids in the finished beer.
- Many attempts have been made to extract the useful hop ingredients, to convert them into a soluble form and to introduce them, as nearly as possible quantitatively, into the beer. The problem is that there are many hop components which contribute to the full quality of beer, and others which are undesirable for one reason or another.
- Rigby (Canadian Patent No. 619,563) described a method of separating a hop extract into its individual chemical components by a lengthy sequence of countercurrent liquid/liquid extractions using buffered aqueous alcohols and hydrocarbons. We have not found the system proposed by Rigby to be economically practical, and much work has been done in recent years to find a simplified system capable of quantitatively separating all the useful hop ingredients from the undesirable ingredients and transferring the former in the right proportion to the beer.
- We have proposed in our Specification No. 1,161,787 and our Patent Application No. 36469/67 (Serial No. 1,187,789), to extract hops with an organic solvent, separate the α -acids from the β -acids and oils by liquid/liquid extraction between aqueous alkali carbonate and a hydrocarbon solvent and isomerise the α -acids to iso α -acids by heating in alkaline solution.
- We have now discovered that small quantities of certain hard resins, including as principal constituents xanthohumol and isoxanthohumol, pass into the aqueous alkaline phase with the α -acids and are responsible for two undesirable effects. Firstly these hard resins (hereinafter referred to for convenience as xanthohumols) tend to separate during the alkali extraction to form a resinous phase which is soluble in neither the hydrocarbon nor the aqueous alkali, entraining a significant proportion of the α -acids, which are thereby lost from the extract. Secondly, part of the xanthohumol remains in the extract and tends to cause cloudiness on addition to beer. The cloud entrains a part of the iso α -acids which is lost if the beer is filtered.
- The xanthohumols referred to herein comprise xanthohumol itself, which is a yellow crystalline solid melting at 172°C having the formula I,



and isoxanthohumol which is formed from xanthohumol in dilute alkali, and has the formula II



Xanthohumols occur in hops to the extent of about 0.9% in fresh and 0.3% in dried hops, based on the total weight of hops.

We have found that hop extracts containing α -acids may be improved if the xanthohumols are separated from the α -acids, preferably prior to any isomerisation thereof. We have discovered that this is most readily achieved by a liquid/liquid extraction, the extract being dissolved in a water-immiscible solvent and the solution contacted with an aqueous solvent (such as 70 to 80% v/v aqueous methanol) which selectively extracts xanthohumol.

Our invention provides a method of preparing bittering adjuncts for beer which comprises (a) preparing a solvent extract of hops containing the hop resins and oils (b) separating xanthohumols from the extract (c) separating the extract into a α -acid-containing fraction substantially free from β -acids and essential oils and a β -acid- and oil-containing fraction substantially free from α -acids and (d) isomerising the α -acids.

Preferably, the solvent extract is prepared by contacting ground oast dried hops or lupulin with a suitable extracting solvent such as benzene or light petroleum, or by any other convenient method as described, for example, in our aforesaid Patent Applications.

The xanthohumols are preferably separated from the extract by contacting a solution of the latter in a first, relatively non-polar organic solvent, with a second relatively polar-solvent which is immiscible with the first solvent and which tends selectively to extract xanthohumols from the solution in the first solvent.

The first solvent is preferably a hydrocarbon, especially petroleum ether or other paraffinic liquid hydrocarbon. Aromatic hydrocarbons such as benzene and toluene are operative but are less preferred. Halogenated hydrocarbon solvents such as chloroform and carbon tetrachloride may be employed, but are less satisfactory than hydrocarbons.

The second solvent is preferably an aqueous lower alcohol, in particular aqueous methanol. Aqueous methanol containing from 70 to 80% v/v of methanol has been found to be particularly efficient. The solution of extract in the first solvent is stirred, shaken or otherwise contacted with the second solvent either batchwise or in a continuous countercurrent column. Preferably the second solvent, after contacting with the first is washed with an immiscible relatively non-polar solvent to recover any α -acids that may have been extracted with the xanthohumols. Conveniently the solvent used for washing may be used as, or as part of, the solvent used to extract the hops, or as or as part of the first solvent. According to a particularly preferred embodiment the hops are extracted with benzene, the resulting extract is recovered and dispersed in light petroleum, insoluble matter is discarded and the dispersion in petroleum is contacted with a countercurrent stream of 70—80% v/v aqueous methanol, the methanol is separated from the petroleum solution and washed with fresh light petroleum which is subsequently used for the treatment of further quantities of benzene extract. The pH of the methanol should not be high enough to extract α -acid salts from the water-immiscible phase.

After separation of the xanthohumols, the solution of extract may be contacted with aqueous sodium or potassium carbonate to separate the α -acids from the β -acids and hop oils. Preferably the α -acids are separated and isomerised by the methods described in our aforesaid Patent Applications.

By our present invention we may provide an isomerised extract comprising the iso α -acids (preferably as their sodium or, better still, potassium salts) together with certain other resins which may be of use in brewing, but substantially free of the oils and of several important classes of resin which tends to reduce the solubility and/or detract from the flavour of the extract, in particular β -acids, xanthohumols and also fixed and essential oils. It is possible to convert certain of these compounds into forms which may be blended

with the isomerised extract. We prefer, however, to recover the extract containing β -acids and essential oils from the first solvent, and free the extract from fixed oils and other undesirable constituents, for example by dissolving the β -acids and essential oils in aqueous methanol, and use the resulting extract as a kettle adjunct for addition to wort prior to fermentation. The isomerised bittering adjunct may conveniently be dissolved in water to form a concentrated alkaline solution of iso α -acid salts, substantially free of xanthohumols and β -acids, which solution is preferably added directly to fermented beer.

A typical extract prepared according to our invention comprises from 10 to 70% (preferably 30 to 50%) by weight of iso α -acids as their sodium or preferably potassium salts but is substantially free from xanthohumols (e.g. not more than 0.2% of xanthohumols, preferably 0.01 to 0.1% by weight). Preferably the extract is also substantially free from β -acids and hop oils. The extract may contain a proportion of water up to 85%, preferably 20 to 50% by weight e.g. 30 to 45%, and/or soft resinous material and any minor components of the hop, which are innocuous.

Separation of xanthohumols according to the present invention increases the utilisation of α -acids and reduces the tendency of the isomerised extract to cause cloudiness in the finished beer.

According to a further aspect, our invention provides a method of purifying an isomerised aqueous solution of alkali metal iso α -acids which comprises contacting the solution with a water-immiscible solvent which is sufficiently polar to extract the iso α -acid from the aqueous solution.

Extracts obtained in this way are purer and more mobile than the extracts obtained by evaporating the aqueous solution directly.

Preferably the iso α -acids are obtained by extracting a solution of hop extract in a water-immiscible non-polar solvent with aqueous alkali (especially aqueous sodium or potassium carbonate), transferring the α -acid salts from the extracting solution into a more concentrated alkaline solution (for example by precipitation with calcium or preferably extraction with a polar solvent as described in our Application No. 48866/65 (Specification No. 1,161,787)), and heating the more concentrated solution to isomerise the α -acids.

Alternatively, an aqueous alkaline solution of α -acids may be boiled to isomerise the α -acids and may be concentrated under vacuum.

The water-immiscible solvent which is sufficiently polar to extract the α -acids from aqueous solution is usually an alcohol, ketone, or carboxylic acid ester and may be, for example, methyl, isobutyl ketone, n-butanol, ethyl acetate or isopropyl acetate.

Preferably the aqueous solution is shaken

with the polar solvent, or contacted in a countercurrent column, and the polar solvent is then evaporated under vacuum, water being added during the evaporation to provide a liquid extract which may most conveniently contain from 30 to 45% by weight of water.

Preferably the potassium salt is recovered, and the pH of the aqueous solution is maintained at about 8.5 to 10.5 during the extraction by addition of potassium carbonate. The aqueous alkaline solution from which the iso α -acids are separated in accordance with this second aspect of the invention preferably has a concentration of from 15 to 40% w/v total solids in solution, e.g. 30% w/v.

EXAMPLE I

Crude hop extract (100 lb) containing 27.8% α -acids, obtained by extracting dried hops with benzene, was dissolved in light petroleum (B.R. 75–95°C) (50 gallons). The insoluble material (6 lb) was discarded. The solution was thoroughly agitated with 75% v/v methanol (10 gallons) for 10 minutes at 50°C. After standing for 1 hour the lower methanol layer was run off. The washing process was repeated with a further quantity of 75% v/v methanol (10 gallons). The methanol washings were combined and agitated with clean light petroleum (6 gallons). After separating the upper petroleum layer was added to the main washed petroleum solution. The lower methanol layer was subjected to distillation to recover the methanol for re-use and yielded a resinous residue (4 lb) rich in xanthohumol. The petroleum solution of the hop resins was then introduced into continuous liquid/liquid extractor column at a flow rate of 10 gallons per hour with a counter flow of 2½% w/v potassium carbonate solution at a rate of 20 gallons per hour. The emergent aqueous alkaline solution (pH 9.0) was introduced continuously into a smaller packed column against a counter flow of clean light petroleum at a flow rate of 1½ gallons per hour. The combined petroleum solutions, containing β -acids, soft resins, fixed and essential oils, were subjected to distillation to recover the solvent, leaving the lupulones etc. for further treatment if desired.

The aqueous solution (80 gallons) contained 3.3% w/v α -acids as potassium salts and was virtually free of β -acids and other undesirable hop constituents. This solution was then introduced into a further countercurrent extractor against a counter flow of n-butanol. The butanol solution (26 gallons) obtained, contained 9.8% w/v α -acids. This solution was subjected to distillation under reduced pressure to recover the butanol. Water was added as necessary during the distillation to displace solvent while maintaining the still temperature below 60°C. The residual aqueous solution (12½ gallons) containing 19.0% w/v α -acids, was boiled for 90 minutes at

atmospheric pressure while maintaining the pH at 10.2 by addition of a 25% w/v aqueous solution of potassium carbonate, to isomerise the α -acids. The resultant solution (10½ gallons) contained 21.3% w/v iso α -acids. This solution was then extracted by agitation with three successive quantities of ethyl acetate (6 gallons, 2+2 gallons). The combined ethyl acetate solutions were subjected to distillation at slightly reduced pressure, with addition of small quantities of distilled water as necessary to displace the solvent.

The residue which constituted the finished isomerised hop extract weight 48½ lb and contained 45.3% w/w iso α -acids. Representing a recovery of 79% in the starting material.

EXAMPLE II

Crude hop extract (300 lb) containing 41.0% α -acids, obtained by extracting dried hops with benzene, was dissolved in light petroleum (B.R. 75–95°C) (120 gallons). The insoluble material (12 lb) was discarded. The solution was passed at a flow rate of 10 gallons per hour through a countercurrent column against a counter flow of 75% v/v methanol at a rate of 8 gallons per hour. The emergent methanol solution was passed continuously to a second smaller column through which it flowed against a counter flow of clean light petroleum at a rate of 2 gallons per hour. The methanol solution emerging from this second column was subjected to distillation to recover the methanol, leaving a residue (20 lb) rich in xanthohumol. The light petroleum from second column was reserved and used later to dissolve a further quantity of hop extract.

The original light petroleum solution from 1st column was passed continuously through a third column against a counter flow of 2½% w/v aqueous potassium carbonate at a rate of 20 gallons per hour, but this rate was controlled such that the emergent aqueous solution had a pH within the range 8.5 to 9.0. The light petroleum emergent from third column was subjected to distillation to recover the solvent, leaving a residue (101 lb) rich in β -acids, hop essential and fixed oil. The aqueous phase was passed continuously through a fourth column against a counter flow of clean light petroleum at a rate of 2 gallons per hour. The light petroleum solution from this fourth column was reserved for later use in dissolving more hop extract. The aqueous phase (265 gallons) containing 4.2% w/v α -acids was collected and after warming to 40°C was treated with a 25% aqueous solution of calcium chloride (20 gallons) until precipitation of the calcium salts of the α -acids was complete. The precipitate was collected on a vacuum filter, the filtrate being discarded. The moist filter cake was dissolved by stirring in 97% v/v methanol (200 gallons). To this mixture was then added

50% w/v aqueous potassium carbonate solution in sufficient quantity to raise the pH of the methanolic solution to 12.2. The solution was then passed through a clarifying filter to remove insoluble calcium carbonate and/or bicarbonate. To the filtrate was added a 50% aqueous solution of citric acid, sufficient to reduce the pH to 9.0. The solution was then subjected to distillation to recover the methanol, leaving an aqueous solution (20 gallons) of potassium salts of α -acids. This solution was diluted to a volume of 60 gallons with distilled water and the pH adjusted to 10.2 by the addition of 50% aqueous potassium carbonate, then boiled under atmospheric pressure until isomerisation was complete. The resultant solution of the potassium salts of the iso α -acids was then concentrated under vacuum to give the isomerised hop extract (180 lb) containing 48.4% iso α -acids, representing a recovery of 71% of the α -acids present in the starting material.

EXAMPLE III

Isomerised hop extract (90 lb) containing 48.4% iso α -acid prepared according to method given in Example 2, was further purified as follows. The extract was dissolved in distilled water (15 gallons) and the solution extracted with methyl isobutyl ketone, three times (5, 2+2 gallons) by mechanism agitation followed by settling and separation. The aqueous layer was discarded. The methyl isobutyl ketone solution of isomerised hop extract was subjected to distillation under reduced pressure to recover the solvent. Water was added during the distillation in sufficient quantity to assist the removal of the isobutyl ketone. The residue, a clear fluid extract (101 lb) containing 41.8% iso α -acids was further diluted with distilled water (4 lb) to give a standardised extract (105 lb) containing 40% iso α -acids and 35% of water.

The xanthohumol content of the above extracts was determined by the following method:

Approximately 1 g. of the extract was weighed accurately and dissolved in distilled water (20 ml.). The solution was transferred to a separating funnel, acidified strongly (pH 1) with hydrochloric acid, and extracted with chloroform (3×5 ml.). The combined chloroform extracts were filtered through a small plug of cotton wool supporting a small quantity of anhydrous sodium sulphate. The filtrate was collected in a 25 ml. graduated flask and diluted to volume with benzene.

A column of silica gel (Merck 0.05–0.2 mm.) 6 cm. long by 6 mm. diameter was prepared by dry packing. 2 ml. of the prepared solution was added to the top of the column and eluted with a mixture of benzene and chloroform (9:1); the eluate (about 40 ml.) was discarded. Elution was continued with pure methanol and the eluate collected

in a 25 ml. volumetric flask. 0.5 ml. N/10 sodium hydroxide was added, and the solution made up to volume with methanol. The optical density of this solution was measured at 438 μ in a 1 cm. cell with methanol as reference.

Calculation:

$$\% \text{ xanthohumol} = \frac{\text{O.D}}{4} \times \frac{25}{2} \times \frac{100}{1000w}$$

- Where W=weight of extract taken (equation derived from fact that 1 mg. xanthohumol in 25 ml. alkaline methanol has an optical density of 4).

WHAT WE CLAIM IS:—

1. A method for preparing bittering adjuncts for beer which comprises (a) preparing a solvent extract of hops containing the hop resins and oils; (b) separating xanthohumols from the extract; (c) separating the extract into a α -acid-containing fraction, which is substantially free from β -acids and hop oils, and a β -acid- and oil-containing fraction which is substantially free from α -acids, and (d) isomerising the α -acid containing fraction.
2. A method according to Claim 1 wherein the solvent extract of hops is dissolved in a first, relatively non-polar, organic solvent and the solution is contacted with a second, relatively, polar, organic solvent which is immiscible with the first solvent and which tends selectively to extract xanthohumols from the solution in the first solvent.
3. A method according to Claim 2 wherein the first solvent is a hydrocarbon.
4. A method according to Claim 3 wherein the first solvent is petroleum ether.
5. A method according to any of Claims 2, 3 and 4 wherein the second solvent is an aqueous lower alcohol.
6. A method according to Claim 5 wherein the second solvent is aqueous methanol.
7. A method according to Claim 6 wherein the second solvent contains from 70 to 80% v/v of methanol.
8. A method according to any foregoing claim wherein the step (c) is effected by contacting a solution of the extract in a water immiscible non-polar solvent with aqueous alkali, separating the aqueous phase from the non-aqueous phase and recovering the α -acids or salts thereof from the aqueous phase.
9. A method according to Claim 8 wherein the aqueous alkali is aqueous sodium or potassium carbonate.
10. A method according to Claim 9 wherein the salts of the α -acids are recovered from the aqueous phase by contacting it with a polar, water-immiscible solvent.
11. A method according to any foregoing claim wherein salts of the α -acids are isomerised according to step (d) by heating in aqueous alkaline solution.
12. A method according to Claim 11 wherein the isohumulate salts are recovered from the aqueous alkaline solution by contact with a polar water-immiscible solvent.
13. A method according to Claim 12 wherein the said polar, water-immiscible solvent in each case is a liquid, water-immiscible alcohol, ketone, or carboxylic acid ester.
14. A method according to Claim 13 wherein the polar, water-immiscible solvent is methyl isobutyl ketone, n-butanol, ethyl acetate or isopropyl acetate.
15. A method according to any of Claims 12 to 14 wherein the pH of the aqueous alkali in each case is maintained between 8.5 and 10.5 whilst it is in contact with the polar, water-immiscible solvent.
16. A method according to any foregoing claim wherein the α -acid salts are isomerised by heating in an aqueous alkaline solution having a concentration of from 15 to 40% w/v total solids.
17. A method according to any foregoing claim substantially as herein described with reference to any of the Examples.
18. Hop extracts whenever prepared by a method according to any foregoing claim.
19. An isomerised hop extract comprising hop resins and containing from 10 to 70% by weight of iso α -acids as their salts but containing less than 0.2% by weight of xanthohumols.
20. An extract according to Claim 19 wherein the xanthohumols are present in a proportion of from 0.01 to 0.1% by weight.
21. An extract according to either of Claims 19 and 20 wherein the iso α -acids are present in a proportion of from 30 to 50% by weight.
22. An extract according to any of Claims 19 to 21 wherein the iso α -acids are present as potassium salts.
23. An extract according to any of Claims 19 to 22 which is substantially free from β -acids and hop oils.
24. An extract according to any of Claims 19 to 23 which contains from 20 to 85% by weight of water.
25. An extract according to any of Claims 19 to 24 wherein the proportion of water is from 30 to 45% by weight.
26. A method of flavouring wort or fermented beer which comprises adding thereto

an extract according to any of Claims 18 to 25.

27. Beer whenever prepared by the method of Claim 26.

Agent for the Applicants,
G. A. BLOXAM,

Chartered Patent Agent,
1 Knightsbridge Green, London SW1X 7QD.

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